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(54) Title: ANTI-TUMOR PREPARATION COMPRISING INTERLEUKIN-2 AND HISTAMINE, ANALOGS THEREOF OR H_2-RECEPTOR AGONISTS

(57) Abstract

A preparation or system for inhibiting the development of malignant tumors and the formation of metastases of malignant tumor cells comprises a first composition comprising an agent selected from the group consisting of histamine, analogues thereof having H_2-receptor activities, endogenous histamine releasing preparations and H_2-receptor agonists, and a second composition comprising IL-2, said first and second compositions being either mixed in a preparation or provided in separate doses in an amount sufficient for treatment of tumors and metastases of malignant tumor cells.

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ANTI-TUMOR PREPARATION COMPRISING INTERLEUKIN-2 AND HISTAMINE, ANALOGS THEREOF OR H_2-RECEPTOR AGONISTS

Technical Field

This invention relates to the field of anti-tumor therapy, and more particularly to the treatment of malignant tumors with interleukin-2 (IL-2). The improvement provided by the present invention is the coadministration of the IL-2 with an agent such as histamine, analogs thereof having H_2-receptor activities, endogenous histamine releasing preparations or H_2-receptor agonists. Unexpectedly potentiated effects are observed in the killing of tumor cells by components of immune system and the prevention or inhibition of metastases of tumor cells.

Background Art

Histamine has been shown to suppress a variety of immune effector mechanism in vitro. This property of histamine is H_2-receptor associated. This effect has been described in the literature as being either directly or indirectly mediated. The direct effect is exerted via the CAMP-mediated suppression of immunocompetent cells. The indirect effect is mediated via the formation of histamine-induced suppressive proteins by suppressor T cells (see, Beer, D.J. et.al, Adv. Immunol. 35: 209 (1984)).

The concept that histamine may provide a suppressive signal for immune effector cells has also provided the background for other types of studies. One example is the testing of the potential antineoplastic effect of cimetidine and other H_2-receptor blockers, alone or in combination with other antineoplastic agents. Results of tests on the effects of these agents on tumor formation which have been conducted in rodents and humans are, however, conflicting. On one hand,

the administration of H_2 blockers has been reported to suppress tumor development in rodents and human subjects (see, e.g., Osband, M.E. et.al, Lancet 1(8221): 636 (1981). Other studies, on the other hand, report that the same treatment enhances tumor growth and even induces tumors (see, e.g., Barna, B.P. et.al, Oncology 40: 43 (1983)).

Histamine has also been shown to suppress rather than enhance the growth and occurrence of several types of tumors (see, e.g., Burtin, C. et.al, Cancer Lett. 12: 195 (1981)). The mechanism for the anti-tumor effects of histamine is not known but has been attributed to H1 receptor activity (see, e.g., Lespinats, G. et.al Br. J. Cancer 50: 545 (1984)). Again, contradictory data exist in this area as well. Histamine, for instance, has been reported to accelerate tumor growth in rodents (Nordlund J.J. et.al J. Invest. Dermatol 81: 28 (1983)).

Interleukin-2 (IL-2) is a lymphokine which has been ascribed a pivotal role in the expansion of T cells in response to antigen (Smith, K.A. Science 240: 1169 (1988)). IL-2 has been shown to exert anti-tumor effects in rodents (see e.g., Lotze, M.T. et.al, in "Interleukin 2", ed. K.A. Smith, Academic Press Inc., San Diego, CA p. 237 (1988), Rosenberg, S., Ann. Surgery 208: 121 (1988)). IL-2 has also been shown to induce partial regression of established tumors in patients with different types of cancer (Rosenberg, S.A. Ann. Surgery 208: 121 (1988)). The anti-tumor effect of IL-2 is potentiated when the compound is given together with autologous lymphocytes which have been cultured in vitro with IL-2 and subsequentially been reinfused to the patient (lymphokine-activated killer (LAK) cells) (Rosenberg, S.A., Ann. Surgery 208: 121 (1988)). This effect is seen both in rodents and in humans. When used in human anti-cancer trials, IL-2 is usually given at very high doses to human tumorbearing subjects and has been reported to induce serious side effects, including renal disturbances, anemia,

reduced platelet counts, and cardiorespiratory effects. In several of these trials the H_2-receptor antagonist ranitidine was used to prevent IL-2 induced dyspepsia and nausea (Rosenberg, supra).

NK cells are considered to play an important role in a host's defenses against arising neoplasms as well as against metastases (Hanna, N., Sur. Synt. Pathol. Res. 2: 68 (1983); Hanna, N. Biochim. Biophys. Acta 780: 213 (1985)). Activation of NK cells, in turn, is known to increase a host's resistance against tumor cells (see, e.g., Lotze, M.T. et.al., supra).

The following are individual in vitro effects of histamine and IL-2 on the regulation of human NK cells known at the time of this invention.

(1) Histamine augments human NK cell cytotoxicity (NKCC) via H_2-receptors.

Histamine, at concentrations of 10^{-4}-10^{-6} M, has been shown to strongly augment the NKCC of human mononuclear cells (MNC) against K562 leukemic cells. The effects is noted both when the effector cells used are unfractionated MNCs or cells enriched for large granular lymphocytes (LGL) by Percoll density gradient centrifugation. The NK-augmenting response to histamine is also mimicked by the H_2-receptor agonist dimaprit with similar potency and efficacy. Two structural analogues to dimaprit, nor-dimaprit and N-methyl-dimaprit, both lacking activities at H_2-receptors, proved to be ineffective under the same test conditions. The NK-augmenting effects of histamine and dimaprit were to be completely antagonized by the H_2-receptor antagonists ranitidine and cimetidine. The NK-augmenting effect of histamine was shown to require the presence of monocytes. In the absence of monocytes, histamine had no effect or weakly suppressed NKCC at the histamine concentrations mentioned. (Hellstrand. K.,

et.al, J. Immunol. 137: 656 (1986)).

(2) Histamine suppresses NK cell activity via T cells.

In contrast to the above-mentioned NK cell activation induced by histamine in the presence of monocytes, histamine has been reported to suppress NKCC against K 562 cells in the presence of T lymphocytes. Thus, in vitro treatment of human T cells with histamine (10^{-3} - 10^{-8}M) induces the production of a soluble factor, histamine-induced soluble suppressor factor (HISSF) that inhibited NK cell cytotoxicity. NK cells alone do not produce HISSF. Production of HISSF induced by histamine is blocked by cimetidine but not by an H_1-receptor antagonist. The inhibition of NK cell cytotoxicity by HISSF is reduced by the addition of IL-2 (6.4-64 U/ml) or interferon-α (500 U/ml) (Nair, M.P.N. et.al., J. Immunol. 136:2456 (1986)). Further, it has been shown that the T-cell mediated suppressive effect of histamine on NK-cell related cytotoxicity is more pronounced in the presence of IL-2 (Welt, S. et.al., Proc. Annu. Meet. Am. Soc. Clin. Oncol. 7:A632 (1988)).

(3) Enchancement of NK cell cytotoxicity by IL-2.

IL-2 rapidly and effectively augments the cytotoxicity of isolated human NK cells in vitro over a broad range of concentrations. The effect has been described both with natural and recombinant forms of IL-2 (Dempsey, R.A., et.-al., J. Immunol. 129:2504 (1982); Phillips, J.H., et.al., J. Exp. Med. 170:291 (1989)). The NK-augmenting effect of IL-2 is related to a cellular IL-2 receptor (IL-2R), p 75 (IL-2Rα) which is expressed on human NK cells (Siegel, J.P. Science 238:75 (1987); Phillips, J.H., et.al., supra). The effect of IL-2 on NK cells is of relevance for the anti-tumor effect induced by this compound since depletion of NK cells from mice was reported to eliminate anti-tumor effects induced by IL-2 treatment (Lotze, M.T., et.al., supra).

In view of the high incidence of cancer in the human population and the at best partial success obtained at present with the different therapies in existence, there is still a need for further improved methods of treating tumors in humans.

Disclosure of the Invention

This invention relates to a preparation or system for inhibiting tumor growth and the formation of metastases of malignant tumor cells comprising a first composition comprising IL-2 and a second composition comprising an agent selected from the group consisting of histamine, analogues thereof having H_2-receptor activities, endogenous histamine releasing preparations and H_2-receptor agonists, said first and second compositions being either mixed in a preparation or provided in separate, doses in an amount sufficient for treatment of tumors and metastases of malignant tumors.

A more complete appreciation of the invention and many of the attendant advantages thereof will be readily perceived as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying figure.

Brief Description of the Drawing

The sole figure is a histogram showing the number of lung metastatic foci of B16 melanoma cells produced by various treatments of male mice. The treatments were conducted with a vehicle (c, control), 25 mg/kg histamine (h), $6x10^3$ U/kg human recombinant IL-2 (IL), 25 mg/kg histamine + $6x10^3$ U/kg human recombinant IL-2 (h+IL), 25 mg/kg ranitidine (r), $6x10^3$ U/kg human recombinant IL-2 + 25 mg/kg ranitidine (r+IL). The compositions were injected to 4-6 week old male Swiss albino mice and $1.5x10^5$B16 melanoma cells were injected i.v.

to the mice 24 hours later. Treatment with vehicle, histamine, IL-2, ranitidine, histamine + IL-2, and rantidine + IL-2 was repeated 1 week after tumor inoculation. The lung mestatic foci (LMF) were monitored after sacrifice of the animals 21 days later. Open bars represents the mean number of LMF on the lung surface calculated from 10 animals per treatment. Similar results were obtained in two separate experiments. The filled bars show lung weights of the respective treatment groups. The weights of lungs correlated to the number of LMF. A seen in the figure, the lung weight of animals treated with histamine + IL-2 was equal to that of normal, tumorfree lungs.

Other objects, advantages and features of the present invention will become apparent to those skilled in the art from the following discussion.

Best Mode for Carrying out the Invention

This invention arose from the unexpected in vitro findings that

(i) IL-2 can suppress NKCC in the presence of monocytes, and
(ii) histamine and IL-2 act synergistically with respect to NKCC enchancement.

These findings prompted the inventors to analyze the in vivo effects of combined histamine/IL-2 treatment on the formation of lung metastases in a mouse animal model.

Unexpectedly, a combined histamine/IL-2 treatment completely prevented metastases of malignant tumor cells when the compounds were given as a single dose 24 hrs prior to and one week after tumor cells inoculation. These are unexpectedly superior results since under similar circumstances neither IL-2 alone nor histamine alone had such beneficial effect. The doses of IL-2 used in the animal experiments

were substantially lower than amounts used in general for treatment of cancer. This is of particular importance since the potentiation of the anti-tumor effect of IL-2 induced by concomitant treatment with histamine permits a reduction of the high doses of IL-2 which are used in cancer therapy. Such high-dose IL-2 treatment is associated with serious side-effects (Rosenberg, S.A., supra).

Provided herein is a preparation or system for inhibiting tumor growth and the metastases of malignant tumor cells in a subject carrying the cells comprising a first composition comprising an agent selected from the group consisting of histamine, analogues thereof having H_2-receptor activities, endogenous histamine releasing preparations and H_2-receptor agonists and a second composition comprising IL-2; said agent and said IL-2 being either mixed in a preparation or provided in separate doses in an amount sufficient for treatment of tumors and metastases of malignant tumor cells.

Analogs of histamine having H_2-receptor activities which are suitable for use in this invention are known in the art and need not be described herein. By means of example, the analogs may hav a chemical structure similar to that of histamine but be modified by the addition of moieties which do not negatively interfere with their histamine-like activities, and in particular with their H_2-receptor activities. Examples of H_2-receptor agonists suitable for use in this invention are those such as dimaprit but not N-methyl-dimaprit or nor-dimaprit. Endogenous histamine releasing preparations suitable for use herein are known in the art. Examples of preparations capable of releasing endogenous histamine are these comprising other lymphokines such as IL-3 or allegens. However, other knwon preparations are also suitable.

IL-2 and compounds such as histamine, analogs thereof, endogenous histamine releasing preparations and H_2-receptor

agonists can be administered separately or in the same composition. The administration can be attained by routes which are known in the art for these compounds and preparations. By means of example they can be administered by local or systemic injection, or infusion, as is known in the art. However, other means of adminstration are also suitable.

The present compounds may also be adminstered by the intraperitoneal and other parenteral routes. Solutions of the active compound as a free acid or a pharmaceutically-acceptable salt may be administered in water with or without a surfactant such as hydroxypropyl cellulose. Dispersion are also contemplated such as those utilizing glycerol, liquid polyethylene glycols and mixtures thereof and oils. Antimicrobial compounds may also be added to the preparations. Injectable preparations may include sterile aqueous solutions or dispersions and powders which may be diluted or suspended in a sterile environment prior to use. Carriers such as solvents or dispersion media containing, e.g., water, ethanol polyols, vegetable oils and the like, may also be added. Coatings such as lecithin and surfactants may be utilized to maintain the proper fluidity of the composition. Isotonic agents such as sugars or sodium chloride may also be added as well as products intended for the delay of absorption of the active compunds such as aluminium monostearate and gelatin. Sterile injectable solutions are prepared as is known in the art and filtered prior to storage and/or administration. Sterile powders may be vacuum dried or freeze dried from a solution or suspension containing them.

Any material added to the pharmaceutical composition should be pharmaceutically-acceptable and substantially non-toxic in the amounts employed. Sustained-release preparations and formulations are also within the confines of this invention.

Pharmaceutically-acceptable carriers as utilized in the context of this patent include any and all solvents, dis-

persion media, coatings, antimicrobial agents, isotonic and absorption delaying agents and the like as is known in the art. All preparations are prepared in dosage unit forms for uniform dosage and ease of administration. Each dosage unit form contains a predetermined quantity of active ingredient calculated to produce a desired therapeutic effect in association with a required amount of pharmaceutical carrier.

Typically, the agent which encompasses histamine, analogs thereof, endogenous histamine releasing preparations and H_2-receptor agonists may be administered in an amount of about 0.1 to 10 mg/day, preferably about 0.5 to 8 mg/day, and more preferably about 1 to 5 mg/day. However, other amounts may also be administered with Il-2 as can be tailored by a practitioner.

Although in the examples the compounds are administered as a sole dose it is understood that for anti-tumor therapies the compounds may be administered for prolonged periods of time. Typically, the treatment may be administered for periods of up to about 1 week, and even for periods greater than 1 month. In some instance after a period of anti-tumor treatment, the treatment may be discontinued and then resumed once again.

The IL-2 may be administered in an amount of about 1.000 to 300.000 U/kg/day, more preferably about 3.000 to 100.000 U/kg/day, and more preferably about 5.000 to 20.000 U/-kg/day, or otherwise as known in the art.

A daily dose may be administered as one dose or it may be otherwise divided into several doses if negative effects are observed.

In one preferred embodiment of the method, the histamine, analogs thereof having H_2-receptor activities, endogenous histamine releasing preparations or H_2-receptor agonists and

the IL-2 are administered on the same days. A still more preferred embodiment of the method of the invention is one wherein the agent is histamine and the histamine is administered in the same composition with IL-2.

In another aspect of the invention it is provided herein a method of increasing the anti-tumor cell effect of IL-2 in a subject comprising co-administering to the subject a first composition comprising IL-2 and a second composition comprising an agent selected from the group consisting of histamine, analogues thereof having the H_2-receptor activities, endogenous histamine releasing preparatios and H_2-receptor agonists; the agent and the IL-2 being administered in amounts and for a period of time effective to attain the desired effect.

As in the case of the prior method the agent and the IL-2 may be administered separately or as a single composition. Typically, the agent is administered in an amount of about 0.1 to 10 mg/day, more preferably about 0.5 to 8 mg/day, and more preferably about 1 to 5 mg/day for a period of time of about 1 week to 1 month, and in some instances for a period greater than 2 months. The Il-2 may be administered in an amount of about 1.000 to about 300.000 U/kg/day, more preferably about 3.000 to 100.000 U/kg/day, and more preferably about 5.000 to 20.000 U/kg/day, for a period of about 1 week to 1 month, and in some cases the treatment may be prolonged for a period greater than about 2 months. The treatment with the two compounds may be discontinued for a period of time and then resumed as was described above. Other regimes and amounts may also be utilized.

Also provided herein is an improvement on a knwon method of treating a subject carrying a malignant tumor with a composition comprising IL-2, the improvement comprising co-administering to the subject a composition comprising an agent selected from the group consisting of histamine, analogues

thereof having H_2-receptor activities, endogenous histamine releasing preparations and H_2-receptor agonists; the IL-2 and the agent being administered in amounts and for a period of time effective to potentiate the anti-metastatic effect of IL-2.

The agent may be administered in amounts as described above, or as an artisan with skill in the art can determine. Similarly, the IL-2 may be administered in amounts known in the art (higher than prescribed herein), as described herein or as an artisan may determine to be suitable for specific applications. Typically, the agent may be administered for a period of time of about 1 week and in some cases for even longer periods of time. Similarly the IL-2 may be administered for a period of time as is known in the art for specific types of tumors or about 1 week to 2 months, and in many instances for longer periods of time as well.

In a particularly preferred embodiment of the method the agent and the IL-2 are administered on the same days for increased potentiation of their mutual effects.

Also provided herein is an improvement on a method of inhibiting tumor growth and the metastases of malignant tumor cells in a subject carrying the cells with a composition comprising IL-2, the improvement comprising co-administering to the subject a composition comprising an agent selected from the group consisting of histamine, analogues thereof having H_2-receptor activities, endogenous histamine releasing preparations and H_2-receptor agonists, the agent being administered in amounts and for a period of time effective to increase the anti-tumor effect of IL-2 and to prevent the metastases of the cells.

Typically, the agent is administered in an amount of 0.1 to 10 mg/day, more preferably about 0.5 to 8 mg/day and more preferably about 1 to 5 mg/day. The IL-2 is administered as

known in the art or in an amount of about 1.000 to 300.000 U/kg/day, more preferably about 3.000 to 100.000 U/kg/day and more preferably about 5.000 to 20.000 U/kg/day. The two compounds may be administered separately or in the same composition as described above.

In one preferred embodiment the agent and the IL-2 are administered on the same days and as a sole composition. This therapy may be continued for a period of up to about 1 week, and even for periods longer than about 4 weeks. Rest periods flanked by treatment periods may also be utilized.

The present methods may be utilized alone or in conjunction with other anti-cancer therapies as seen suitable by a practitioner.

Having now generally described this invention, the same will be better understood by reference to certain specific examples, which are included herein for purposes of illustration only and are not intended to be limiting of the invention or any embodiment thereof, unless so specified.

Examples

Example 1: In vitro Studies with IL-2 and Histamine.

This example provides a study on the effects of histamine, ranitidine and recombinant IL-2 (25U/ml), alone or in combination, in the NK-cell cytotoxicity (NKCC) of human mononuclear cells (MNC).

The MNC were obtained from pheripheral venous blood of healthy blood donors and recovered by Ficoll-Hypag centrifugation followed by Percoll density gradient fractionation as previously described (Hellstrand, K. et.al. J. Immunol. 137: 656 (1986)). A low density Percoll fraction 8 used in the experiments contained approximately 30% monocytes and

was enriched for large granular lymphocytes (LGL).

NKCC was measured in a ^{51}Cr-release microcytotoxicity assay using K 562 erythroleukemic, Daudi B-lymphoblastoid, Molt-4 T cells, and Chang liver cells as target cells (all malignant cells).

NKCC was determined in sextublicate as specific ^{51}-Cr-release at a MNC:target cell ratio of 15:1 or 30:1. The assays were performed in Iscove's medium containing antibiotics and 10% human AB+ serum. Histamine, IL-2, and ranitidine or various combinations thereof (see Table below) were added at the onset of a 4 hr ^{51}Cr-release assay. Control cells were given vehicle only.

The results obtained were as follows. Histamine (10^{-4} - 10^{-7}M) augmented NK cellcytotoxicity against all types of tumor cells in the presence of monocytes. This effect was entirely blocked by equimolar concentrations of ranitidine. Ranitidine alone did not affect NKCC. In the absence of monocytes, i.e., after removal of monocytes by 1 hr incubation of the MNC on a Petri dish or by carbonyl iron treatment, histamine, ranitidine, or histamine plus ranitidine were devoid of effects at any concentration tested.

IL-2 (5-50 U/ml) alone was unexpectedly ineffective or even suppressed NKCC in the presence of monocytes. After removal of monocytes, IL-2 strongly augmented NKCC dose-dependently over the same range of concentrations. Histamine, ranitidine or histamine plus ranitidine did not affect IL-2-induced enchangement of NKCC in monocyte-depleted MNC. However, histamine plus IL-2 yielded a strong synergistic enchancement of NKCC in the presence of monocytes against all tumor cell targets tested. This synergistic effect was entirely blocked by the presence of ranitidine. Results of a representative experiment are shown i a Table below.

Table: Demonstration of Synergistic Activation of Human NKCC by Combined Treatment with Histamine and IL-2

Treatment[1]	NKCC (cell lysis %) ± s.e.m.) against repective tumor target cells			
	K562	Daudi	Chang	Molt-4
medium	21.6±1.2	3.9±1.1	17.4±1.1	11.8±0.5
Hist(10^{-5}M)	35.9±0.9	12.8±1.0	32.1±2.0	43.2±1.5
IL-2(25U/ml)	12.0±0.7	1.4±0.5	9.8±0.6	5.2±0.4
Ran (10^{-5}M)	20.8±1.9	4.3±0.8	19.7±1.3	13.0±1.0
Hist + IL-2	55.4±1.0	41.4±0.9	59.7±0.6	69.4±3.0
Hist + Ran	20.1±1.4	5.0±1.4	19.4±1.0	13.0±1.1
Ran + IL-2	11.3±1.3	1.9±0.3	10.4±0.9	6.4±0.7
Hist + Ran + IL-2	13.4±2.0	2.0±0.7	10.0±0.5	8.0±1.1

1 Effector MNC were recovered from peripheral blood by Ficoll-Hypaque and Percoll density gradient centrifugation. A low density Percoll fraction with 27% monocytes was used at a final effector to target cell ratio of 15:1 (K562 Chang, and Molt-4) or 30:1 (Daudi).

2 Hist = histamine, IL-2 = interleukin-2
Ran = Ranitidine

Example 2: In vivo Studies Model of Antitumor Effects of Histamine, IL-2, Rantidine and Combinations of these Compunds in a Mouse Tumor Animal Model.

In vivo experiments were carried out with histamine or IL-2 alone, and with combinations of these compunds in a mouse tumor animal model.

Histamine (25 mg/kg), ranitidine (25 mg/kg), and human recombinant Il-2 (6.000 U/kg), alone or in combination, were administered, as a single-dose to 4-6 weeks old male Swiss albino mice (20 g) 24 hours prior to and 1 week after intravenous inoculation of B 16 mouse melenoma cells (150.000 cells/mouse). Each treatment group comprised 10 animals. Twenty-four hours after treatment, NK-cell sensitive B16 mouse melanoma cells (150.000 cells/mouse) were inoculated intravenously. Controls were run with animals treated with the respective drug vehicles.

Lung metastatic foci (LMF) on the surface of the lungs were monitored macroscopically 21 days later. LMF were counted by an unbiased observer using a 10 x magnifying microscope. All LMF visible on the lung surface were counted.

The weights of the lungs were measured immediately after sacrifice of the mice and correlated in a virtually linear fashion to the number of LMF.

Example 3: Results of the Tests Conducted in Example 2.

Under the therapeutic regimen depicted in Example 2, histamine alone was found to relatively effectively reduce the number of LMF. 25 mg/kg of histamine yielded approximately an about 50% reduction whereas 250 mg/kg of histamine yielded an about 80-90% reduction of LMF.

This effect was mimicked by dimaprit with similar potency.

Ranitidine augmented LMF about 100%.

IL-2 alone reduces LMF by about 40-70%.

The combined treatment with histamine (25 mg/kg) and IL-2 completely prevented LMF (see the Figure). None of the animals (n=10) treated with histamine (25 mg/kg) + Il-2 ($6x10^3$ U/kg) displayed visible tumors. None of the animals (n=10) treated with histamine (25 mg/kg) or IL-2 ($6x10^3$ U/kg)

alone were completely free of visible tumors. IL-2 was virtually ineffective in the presence of rinitidine. The lung weights of animals receiving histamine plus IL-2 was equal to the weight of lungs from mice that had not been subject to tumor cell inoculation. Histamine, IL-2 or histamine plus IL-2 was found not to affect lung weight of animals which did not receive tumor cells.

CLAIMS

1. A pharmaceutical preparation or system for inhibiting tumor growth and the metastases of malignant tumor cells, characterized in, a first composition comprising an agent selected from the group consisting of histamine, analogues thereof having H_2-receptor activities, endogeneous histamine releasing preparations and H_2-receptor agonists and a second composition comprising IL-2, said first and second compositions being either mixed in a preparation or provided in separate doses in an amount sufficient for treatment of tumors and metastases of malignant tumor cells.

2. A pharmaceutical preparation or system as claimed in claim 1, characterized in, that the daily dose of said agent is between 0.1 and 10 mg, preferably between 0.5 and 8 mg and more preferably between 1 and 5 mg.

3. A pharmaceutical preparation or system as claimed in claim 1 or 2, characterized in, that the daily dose of IL-2 is between 1.000 and 300.000 U/kg, preferably between 3.000 and 100.000 U/kg and more preferably betweeen 5.000 and 20.000 U/kg.

4. A pharmaceutical preparation or system as claimed in claim 1, characterized in, that the agent is histamine.

5. A pharmaceutical preparation or system as claimed in any of the preceding claims,
c h a r a c t e r i z e d i n,
that it contains one or more pharmaceutically-acceptable carriers such as solvent, dispersion medium, coating, anti-microbial agent, isotonic and absorption delaying agents and the like.

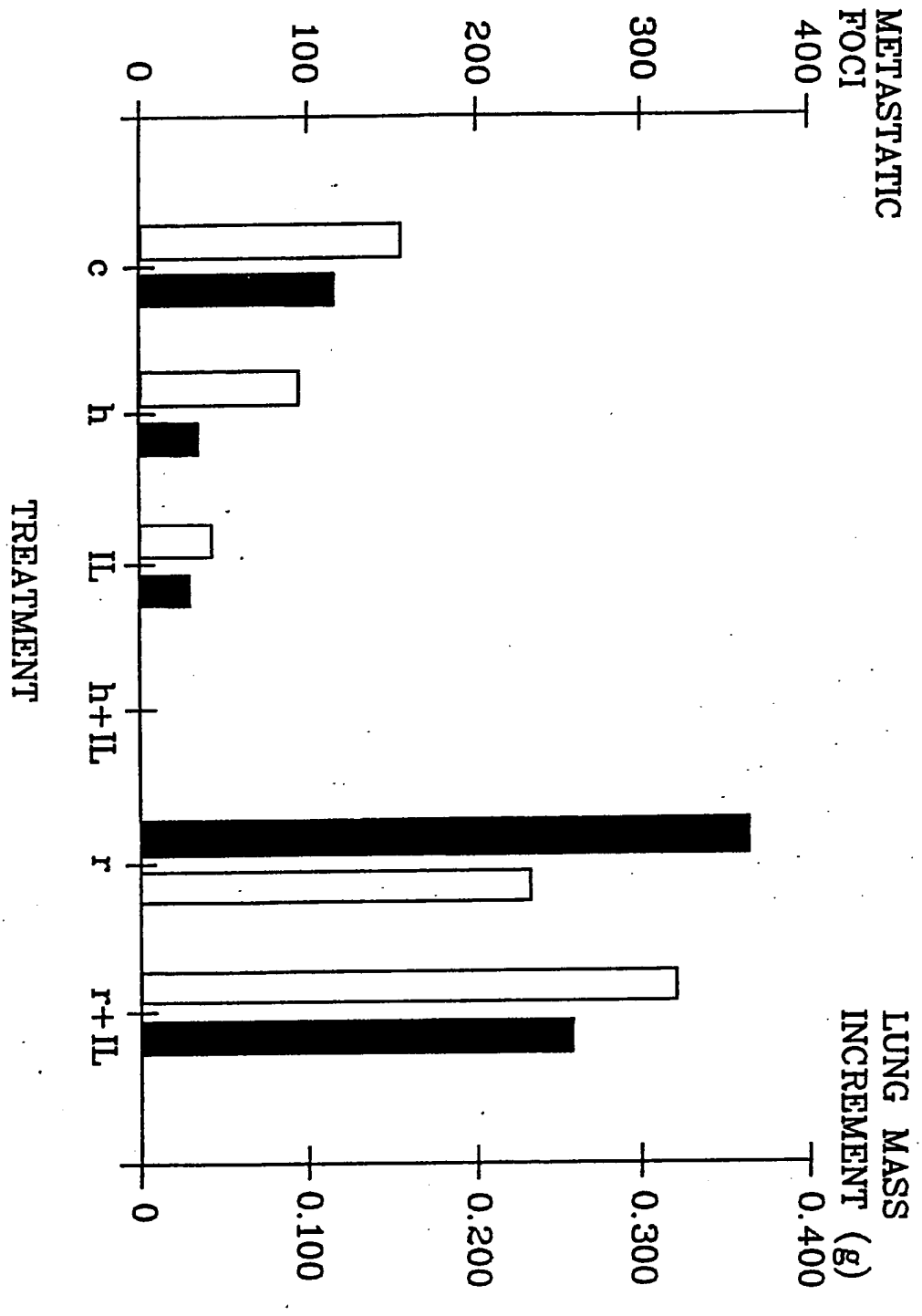
6. Use of a first composition comprising an agent selected from the group consisting of histamine, analogues thereof having H_2-receptor activities, endogeneous histamine releasing preparations and H_2-receptor agonists and a second composition comprising IL-2 for preparing a pharmaceutical preparation or system for inhibiting tumor growth and the metastases of malignant tumor cells, said first and second compositions being either mixed in a preparation or provided in separate doses in an amount sufficient for treatment of tumors and metastases of malignant tumor cells.

7. Use as claimed in claim 6,
c h a r a c t e r i z e d i n,
that the daily dose of said agent is between 0.1 and 10 mg, preferably between 0.5 and 8 mg and more preferably between 1 and 5 mg.

8. Use as claimed i claim 6 or 7,
c h a r a c t e r i z e d i n,
that the daily dose of IL-2 is between 1.000 and 300.000 U/kg, preferably between 3.000 and 100.000 U/kg and more preferably betweeen 5.000 and 20.000 U/kg.

9. Use as claimed in claim 1,
c h a r a c t e r i z e d i n,
that the agent is histamine.

10. Use as claimed in any of claims 6 - 9, characterized in, that it contains one or more pharmaceutically-acceptable carriers such as solvent, dispersion medium, coating, anti-microbial agent, isotonic and absorption delaying agents and the like.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0.200
0.300
0.400

FIG. 1

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE 90/00599

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: A 61 K 37/02, C 07 K 13/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	A 61 K; C 07 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	Chemical Abstracts, volume 104, no. 17, 28 April 1986, (Columbus, Ohio, US), Droege, Wulf et al: "Histamine augments interleukin-2 production and the activation of cytotoxic T lymphocytes ", see page 526, abstract 146898m, & Immunopharmacology 1986, 11(1), 1- 6ä --	1-10
P,A	Dialog Information Services, File 154, Medline 83-91/Jan, Dialog accession no. 07416865, Okamoto H: "Possible involvement of adenosine 3':5'-cyclic monophosphate and extracellular calcium ions in histamine stimulation of interleukin-1 release from macrophage-like P388D1 cells. --	1-10

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* Special categories of cited documents:[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
14th January 1991	1991 -01- 15
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Anneli Jönsson

Form PCT/ISA/210 (second sheet) (January 1985)

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.PCT/SE 90/00599

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the Swedish Patent Office EDP file on 90-11-28
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date